Nuclear Magnetic Resonance Spectroscopy. Application of Pulse and Fourier Transform Carbon-13 Nuclear Magnetic Resonance Techniques to Structure Elucidation. Rauwolfia Alkaloids[1]

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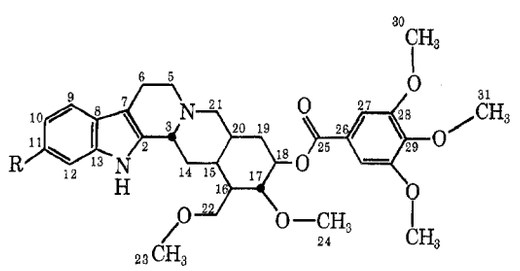
Received October 24, 1972

The natural-abundance pulse and Fourier transform ^{13}C nmr spectra of several Rauwolfia alkaloids have been recorded. Using noise-decoupling, partial single-frequency off-resonance decoupling (SFOR), and lanthanide chelate induced chemical-shift changes (lanthanide shifts), a self-consistent series of assignments have been made for the observed resonances.

We have previously commented on both the vast amount of research which has already been directed toward alkaloid structure elucidation and the possible application of ^{13}C magnetic resonance (^{13}C nmr) spectroscopy to this problem.[4] Our efforts in this area have been continuing and we would now like to report that with the aid of several advances in magnetic resonance techniques, which have been brought to fruition in the last few years, we are now able to record and interpret routinely, with a rather high degree of certainty, the ^{13}C nmr spectra of even rather complex alkaloids.

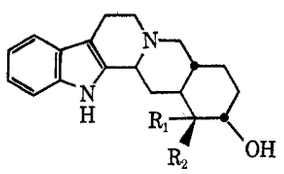
The natural-abundance ^{13}C magnetic resonance spectra of reserpine (I), deserpidine (II), corynanthine



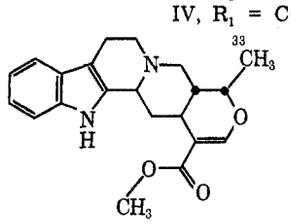
I, R = OCH_3(32)
II, R = H

(III), yohimbine (IV), ajmalicine (V), and reserpinine (VI) have been obtained using pulse and Fourier transform (PFT) techniques.[5] These alkaloids belong to the Rauwolfia family, with compounds I–IV belonging to the yohimbine group, and V and VI to the heteroyohimbine class.

In the majority of instances, the observed resonances could be assigned to specific carbon atoms on the basis of noise-decoupling,[6–8] single-frequency off-resonance



III, R_1 = H; R_2 = CO_2CH_3
IV, R_1 = CO_2CH_3; R_2 = H



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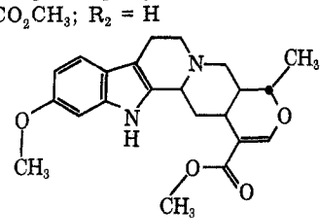


VI

decoupling (SFOR),[9,10] and lanthanide chelate induced chemical-shift changes.[11] Chemical modification of the various alkaloids was, in general, not required, thereby permitting total spectral analysis to be accomplished within a matter of hours. However, formation of simple derivatives such as methyl reserpate from reserpine was found to provide additional information on specific questions of spectral assignments.

A typical, completely proton-decoupled spectrum, that of reserpine, is shown in Figure 1. That the intensities of the peaks in this spectrum do not correspond in a simple way to the statistical numbers of carbons present is a consequence of unequal relaxation times and a short acquisition time for the free-induction decay signal.[5,12] Indeed, the acquisition time was purposely chosen to be sufficiently short so as to result in complete saturation of the signal of the deuteriochloroform used as solvent. This mode of operation is helpful in keeping the solvent peaks from overlapping the alkaloid peaks.

The collected data along with the corresponding assignments for alkaloids I–VI are presented in Tables I–III. Utilization of this data in alkaloid structure elucidation is relatively straightforward. As tabulations of carbon chemical shifts begin to appear with

(1) Supported by the Public Health Service, Research Grant No. GM-11072 from the Division of General Medical Sciences, and by the National Science Foundation.

(2) National Institutes of Health Postdoctoral Fellow, 1971–1972.

(3) NATO Postdoctoral Fellow, 1970–1971.

(4) (a) W. O. Crain, Jr., W. C. Wildman, and J. D. Roberts, *J. Amer. Chem. Soc.*, **93**, 990 (1971); (b) P. W. Sprague, D. Doddrell, and J. D. Roberts, *Tetrahedron*, **27**, 4857 (1971); (c) see G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists," Wiley-Interscience, New York, N. Y., 1972, Chapter 8, for references and discussion of procedures; (d) J. B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York, N. Y., 1972, also offers an excellent treatment of resonance assignments.

(5) T. C. Farrar and E. D. Becker, "Pulse and Fourier Transform NMR," Academic Press, New York, N. Y., 1971.

(6) F. J. Weigert, M. Jautelat, and J. D. Roberts, *Proc. Nat. Acad. Sci. U. S.*, **60**, 1152 (1968).

(7) L. F. Johnson and M. E. Tate, *Can. J. Chem.*, **47**, 63 (1969).

(8) R. R. Ernst, *J. Chem. Phys.*, **45**, 3845 (1966).

(9) H. J. Reich, M. Jautelat, M. T. Messe, F. J. Weigert, and J. D. Roberts, *J. Amer. Chem. Soc.*, **91**, 7445 (1969).

(10) M. Jautelat, J. B. Grutzner, and J. D. Roberts, *Proc. Nat. Acad. Sci. U. S.*, **65**, 288 (1970).

(11) W. D. Horrocks, Jr., and J. P. Sipe, III, *J. Amer. Chem. Soc.*, **93**, 6800 (1971), and references cited therein.

(12) *Cf.*, for example, J. S. Waugh, *J. Mol. Spectrosc.*, **35**, 298 (1970); R. Freeman, K. E. R. Pachler, and G. N. LaMar, *J. Chem. Phys.*, **55**, 4586 (1971).

TABLE I

CARBON-13 CHEMICAL SHIFTS OF RAUWOLFIA ALKALOIDS[a]

Carbon	I	II	III	IV	V	VI	VII
2	61.9	61.8	58.4	58.0	58.1	59.7	58.3
3	131.8	133.0	132.2	132.4	132.5	133.4	
5	143.6	140.1	140.2	139.8	139.3	139.9	
6	175.8	176.8*	171.8	171.1	170.7	171.9	
7	84.8	85.5	86.3	85.4	84.6	85.6	82.9
8	70.4	65.3	66.0	65.4	65.3	71.7	64.2
9	74.2	74.0‡	75.5	74.8	74.5	75.6	74.8
10	83.7	72.1	72.4	71.7	71.2	83.7	71.8
11	36.5	75.4‡	74.5	73.7	73.2	37.3	73.7
12	97.3	82.3	82.0	81.7	81.7	98.5	82.0
13	56.1	57.2	56.4	56.3	56.6	56.1	56.1
14	168.3*,[b]	159.4*,[b]	159.8	158.9	159.7	159.1	
15	160.2†	161.0†	156.2*	156.2	161.9	154.9*	
16	140.9	141.4°	142.1	140.3*	85.8	86.4	
17	114.6	115.5	126.4	125.5	37.9	38.1	
18	114.6	115.5	164.8	160.9			
19	162.9*,[b]	169.4*,[b]	169.3	169.3	118.8	121.1	
20	158.6†	163.8†	158.6*	152.5	151.6	162.0*	
21	138.8	144.0	131.0	131.4	135.7	137.4	
22	19.8	20.3	20.0	17.4	25.0	27.4	
23	140.9	141.6°	142.1	140.9*	141.7	143.7	
24	140.9	141.6°					
25	27.0	27.3					
26	67.3	67.8					
27	85.7	86.2					
28	39.6	39.8					
29	50.2	50.7					
30	136.4	137.4					
31	131.8	133.1					
32	136.9					138.6	
33					177.6	175.4	

[a] All shifts are in parts per million upfield from CS_2. [b] These assignments reflect a greater sensitivity of the C-19 resonances to shift reagents than those of the C-14 carbons. *, ‡, †, ° represent signals where the assignments may be reversed.

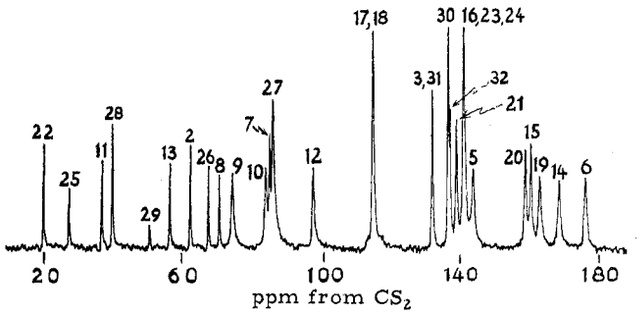
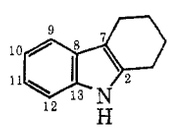


Figure 1.—A PFT ^{13}C nmr spectrum of reserpine (I) in deuteriochloroform. The spectral width was 5000 Hz, 5500 transients, with an acquisition time of 0.4 sec. The plot has 2.5 Hz per spectral data point. The numbers beside each peak correspond to the assignments of Table I.

increasing frequency,[13] it becomes easier to make relatively accurate assignments on the basis of the observed chemical shifts. The ^{13}C nmr signals of the indole rings of compounds II–V could be readily identified by direct comparison to the ^{13}C nmr spectrum of tetrahydrocarbazole (VII). As is shown in Table I, the aromatic and unsaturated resonances of tetrahydrocarbazole display chemical shifts which are very similar to those of the corresponding carbon atoms in II–V, and the sequence of chemical shifts for the carbon atoms in VII is maintained throughout with II–V.



VII

The effect of methoxy groups on ^{13}C nmr shifts has been studied previously.[14] In an extension of this work, we have found the effect in a variety of aromatic substances to be quite regular and predictable. A methoxy substituent shifts a para carbon atom *ca.* 6.8 ppm to higher field, a meta carbon *ca.* 2.4 ppm to lower field, and an ortho carbon *ca.* 15.0 ppm to higher field, as compared to the unsubstituted aromatic compound. If the ortho carbon bears a substituent, then the upfield shift is reduced from 15.0 ppm to *ca.* 8.8 ppm. The methoxylated carbon atom itself is shifted *ca.* 31.3 ppm downfield from where it would come into resonance, were it unsubstituted. If several methoxy groups are present, then their effects are approximately additive. Using deserpidine (II) as a model compound, the chemical shifts for the corresponding carbon atoms in reserpine (I) can be predicted by using the above values (Table IV). The predicted shifts are not highly accurate but the predicted values are in the correct sequence and are accurate enough to facilitate making assignments. The trimethoxybenzoic acid residues in I and II were also analyzed in an analogous fashion with comparable success.

(13) See, for example, P. S. Pregosin and E. W. Randall in "Determination of Organic Structures by Physical Methods," Vol. IV, F. C. Nachod and J. J. Zuckerman, Ed., Academic Press, New York, N. Y., 1971.

(14) P. C. Lauterbur, *J. Amer. Chem. Soc.*, **83**, 1846 (1961); H. Spiesecke and W. G. Schneider, *J. Chem. Phys.*, **35**, 731 (1961).

TABLE II

SINGLE-FREQUENCY OFF-RESONANCE RESULTS[a]

Carbon	I	III	IV	V
2	ST	ST	ST	ST
3	DQ	DQ	DQ	DQ
5	ST	ST	ST	ST
6	ST	ST	ST	ST
7	ST	ST	ST	ST
8	ST	ST	ST	ST
9	DQ	DQ	DQ	DQ
10	DQ	DQ	DQ	DQ
11	ST	DQ	DQ	DQ
12	DQ	DQ	DQ	DQ
13	ST	ST	ST	ST
14	ST	ST	ST	ST
15	DQ	DQ	DQ	DQ
16	DQ	DQ	DQ	ST
17	DQ	DQ	DQ	DQ
18	DQ	ST	ST	
19	ST	ST	ST	DQ
20	DQ	DQ	DQ	DQ
21	ST	ST	ST	ST
22	ST	ST	ST	ST
23	DQ	DQ	DQ	DQ
24	DQ			
25	ST			
26	ST			
27	DQ			
28	ST			
29	ST			
30	DQ			
31	DQ			
32	DQ			
33				DQ

[a] ST = singlet or triplet; DQ = doublet or quartet.

All of the resonances below 130 ppm could be assigned readily to specific carbon atoms, with the SFOR results being used to remove occasional ambiguities or merely to corroborate the postulated assignments. It is to the high-field side of 130 ppm that serious problems in assignment were encountered, as this region contains the majority of the aliphatic carbon resonances which are often methylene carbons, with nonequivalent protons expected to provide further difficulties in the use of the SFOR technique. In order to present some idea of the complexity involved, we note that reserpine (I) has, besides five methylene carbons, six methyne carbons and five methoxy carbons, all of which are different and expected to absorb in this area. It was easy to distinguish the resonances of those aliphatic carbon atoms connected directly to oxygen or nitrogen from those not so substituted. For the latter, the SFOR experiments provided some further clarification but, in general, a variety of unresolved assignments remained. For example, it was not possible to assign the five different methoxy carbons in I to specific signals on the basis of chemical-shift considerations alone.

A partial solution to this dilemma was achieved with paramagnetic lanthanide chelate shift reagents.[11,15] Because the bulk of functional groups in alkaloids I–VI are localized in one portion of the molecule, it was anticipated that, in accord with the $[(3\cos^2\theta - 1)/r^3]$ formalism, the shifts of those carbon atoms located farthest from this complexation area should display the lowest sensitivity to variations in shift reagent concentrations (provided, of course, that θ is small, as it usually seems to be). Carbon atoms located in the immediate vicinity of the complexation site should exhibit an increased sensitivity to chelate concentration, while the other carbons would be expected to display intermediate behavior.

(15) E. Wenkert, D. W. Cochran, E. W. Hagaman, R. B. Lewis, and F. M. Schell, *J. Amer. Chem. Soc.*, **93**, 6271 (1971), and references cited therein.

TABLE III

LANTHANIDE INDUCED CHEMICAL-SHIFT CHANGES[a,b]

Carbon	I	IV	V
2	0.9	0.2	1.8
3	5.3	0.4	2.0
5	0.8	0.0	0.8
6	0.3	0.0	0.9
7	0.1	−0.4[c]	1.0
8	0.3	0.0	0.6
9	0.1	0.0	0.5
10	0.1	0.0	0.4
11	0.3	0.0	0.4
12	0.2	0.0	0.6
13	0.5	0.0	0.9
14	1.5	0.5	5.0
15	1.1	1.0	4.3
16	3.5*	1.2	6.6
17	4.5	3.3	3.0
18	5.5	1.3	
19	1.8	1.1	0.9
20	1.7	0.5	2.0
21	0.9	0.3	1.0
22	2.8	2.2	6.7
23	3.5*	0.9	5.6
24	1.0*		
25	4.4		
26	5.6		
27	5.8		
28	14.9		
29	24.1		
30	8.0		
31	16.6		
32	0.2		
33			0.4

[a] Shifts are in parts per million upfield from the resonance position in the absence of shift reagent. [b] Values are for $\{[Pr(FOD)_3]/[alkaloid]\} = 0.5$. [c] A minus sign indicates a downfield shift. * Represents assignments which may be reversed.

TABLE IV

CALCULATED CARBON 13 CHEMICAL SHIFTS FOR RESERPINE (I)[a]

Carbon	II (model)	I (predicted)	I (observed)
8	65.3	72.1	70.4
9	75.4	73.0	74.2
10	72.1	87.1	83.7
11	74.0	42.7	36.5
12	82.3	97.3	97.3
13	57.2	54.8	56.1

[a] All shifts are in parts per million upfield from CS_2.

Lanthanide shift measurements with reserpine (I) have been particularly illuminating. We present here a few selected results to demonstrate the applicability of the lanthanide shift technique to the ^{13}C nmr spectra of large, multifunctional molecules. The noise-decoupled ^{13}C nmr spectrum of reserpine is shown in Figure 1. Reserpine contains 30 different carbon atoms, 16 of which are expected to come into resonance below 120 ppm. However, only 15 absorptions are present,

with 11 more being apparent above 120 ppm. Thus, there are four carbon atoms which are accidentally equivalent with other carbon atom(s). Gradual addition of the lanthanide chelate, $Pr(FOD)_3$, led to the eventual appearance of 29 of the 30 possible signals. The five methoxy carbons in reserpine (I) were located at 131.8, 136.4, 136.9, and 140.9 (2) ppm by chemical shift, intensity, and SFOR considerations. The signals at 131.8, 136.4, and 140.9 ppm were of greater intensity than most other peaks in the ^{13}C nmr spectrum of I, indicating possible degeneracy. Addition of the shift reagent produced two resonances from the 131.8- and 140.9-ppm signals, the 136.4-ppm absorption still remaining singular and unreduced in intensity. One of the peaks at 131.8 ppm was assigned to a nonmethoxyl carbon (C-3). The high-intensity signal at 136.4 ppm was assigned to the two isochronous C-30 methoxy carbon atoms. Because of its dramatic sensitivity to shift-reagent concentration, the other component of the 131.8-ppm resonance is assigned to the methoxy carbon, C-31. Of all the carbon atoms of I, C-29 displays the greatest sensitivity to lanthanide chelate concentration (Table III) and is followed in this respect by C-31, C-28, and C-30, in that order. The results suggest that the shift reagent complexes with reserpine preferentially, although probably by no means exclusively, at the oxygen which is connected to C-29. Because C-23 and C-24 are closer to the complexation site than C-32, we expect them to have an appreciably greater sensitivity to shift-reagent concentration than C-32 and therefore attribute two of the three components of the 140.9-ppm signal to C-23 and C-24. C-32 is then assigned to the signal at 136.9 ppm which shows, as expected, a diminished sensitivity to shift reagent concentration.

Further application of the above procedures to alkaloids I–VI has led to the other assignments given in Table I, which will not be discussed in detail. It seems clear that PFT ^{13}C nmr will play an increasingly important role in the structural analysis of natural products.

Experimental Section

The ^{13}C spectra were obtained using a "Brukarian" pulsed FT spectrometer which was the previously described[6,16] Varian digital frequency sweep instrument operating at 15.09 MHz, but modified by substitution of a Bruker pulse amplifier, probe, receiver, and internal deuterium lock. The pulses were derived from a Varian pulse box, and the free-induction decay was accumulated and transformed with a 16K Varian 620i computer.[17]

All of the alkaloids, except corynanthine (III), were dissolved in chloroform-*d* to yield 0.5–2.0 *M* solutions. Corynanthine was dissolved in chloroform-*d* containing a small amount of ethanol to enhance solubility. The spectra were referenced to external carbon disulfide by the relationship $\delta_C^{CS_2} = \delta_C^{CDCl_3} + 115.4$ ppm.

Registry No.—I, 50-55-5; II, 131-01-1; III, 483-10-3; IV, 146-48-5; V, 483-04-5; VI, 482-96-2; VII, 942-01-8.

Acknowledgment.—We thank Dr. M. W. Klohs of Riker Laboratories, Inc., for supplying us with samples of many of the Rauwolfia alkaloids used in this research.

(16) F. J. Weigert and J. D. Roberts, *J. Amer. Chem. Soc.*, **89**, 2967 (1967).

(17) We are much indebted to Dr. Bruce Hawkins for his help in the development of this spectrometer system.

Stable Carbocations. CXLIX.[1] Fourier Transform Carbon-13 Nuclear Magnetic Resonance Spectroscopic Study of Protonated Mono- and Dicarboxylic Acid Esters in FSO_3H–SbF_5 Solution

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Received December 27, 1972

The ^{13}C nmr chemical shifts for a series of protonated aliphatic carboxylic acid esters were determined in FSO_3H–SbF_5 solution together with those of their parent esters. Protonation of esters results in deshielding of the carbonyl carbon resonance of the order of 17–21 ppm, of the carbons α to the alkyl oxygen, 12–23 ppm, and those α to the carbonyl group, 0–3 ppm. At the same time generally a slight shielding of most other carbon resonances is observed. The results have been correlated with other substituent effects and with ^{13}C resonances in corresponding hydrocarbons. The pmr and cmr spectra of several protonated diesters in FSO_3H–SbF_5 at $-60°$ have also been studied. The results indicate that dicarboxylic acid esters, including those of oxalic acid, are diprotonated under these conditions.

Several recent instrumental developments in both slow passage and pulsed nuclear magnetic resonance spectrometers[3,4] have allowed for the routine determination of ^{13}C chemical shifts at ^{13}C natural abundance.[5]

The ^{13}C spectra of several carboxylic acids and their tetramethylammonium salts have been recorded in aqueous solution by Hagan and Roberts.[6] Using the INDOR technique[7] in our previous work, we obtained the ^{13}C spectra of protonated formic, acetic, propionic, and benzoic acids in FSO_3H–SbF_5 solution.[8] Carboxylic acid esters and their protonated derivatives have been examined by ^{13}C nmr spectroscopy to a much lesser extent. The carbonyl carbon shifts for a series of carboxylic acid esters have been reported[9] as well as

(1) Part CXLVIII: G. A. Olah and G. Liang, *J. Amer. Chem. Soc.*, **95**, 3792 (1973).

(2) Postdoctoral Research Fellow, 1971–1973.

(3) F. J. Weigert and J. D. Roberts, *J. Amer. Chem. Soc.*, **89**, 2967 (1967).

(4) T. C. Farrar and E. D. Becker, "Pulse and Fourier Transform NMR," Academic Press, New York, N. Y., 1971, pp 34–45.

(5) For example, see (a) J. B. Grutzner, M. Jautelat, J. B. Dence, R. A. Smith, and J. D. Roberts, *J. Amer. Chem. Soc.*, **92**, 7107 (1970); (b) A. J. Jones, D. M. Grant, M. W. Winkley, and R. K. Robins, *ibid.*, **92**, 4079 (1970); (c) J. D. Roberts, F. J. Weigert, J. I. Kroschwitz, and H. J. Reich, *ibid.*, **92**, 1338 (1970); (d) F. J. Weigert and J. D. Roberts, *ibid.*, **92**, 1347 (1970).

(6) R. Hagen and J. D. Roberts, *ibid.*, **91**, 4504 (1969).

(7) E. B. Baker, *J. Chem. Phys.*, **37**, 911 (1962).

(8) G. A. Olah and A. M. White, *J. Amer. Chem. Soc.*, **89**, 7072 (1967).

(9) J. B. Stothers and P. C. Lauterbur, *Can. J. Chem.*, **42**, 1563 (1964).